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COLLEGE OF AGRICULTURE UNIVERSITY OF NEBRASKA
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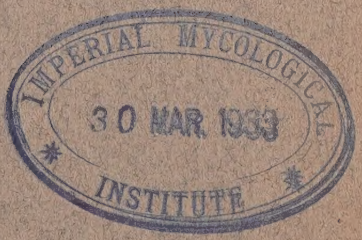
The Nature of Resistance in Alfalfa to Wilt

(*Aplanobacter insidiosum* L. Mc.)

GEORGE L. PELTIER AND F. R. SCHROEDER
Department of Plant Pathology



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SUMMARY

Numerous sections of alfalfa plants of various ages, representing various stages of the wilt disease in both naturally and artificially infected material, have been studied and interpreted in the light of evidence observed in intensive field and greenhouse studies carried on for the past five years. The usual or normal progress of the bacteria from their entry until the death of the plant ensues has been followed.

In the main it has been found that resistance in some alfalfas is associated with certain morphological features, particularly in the root, which inhibit rapid development and invasion of the vital tissues by the bacteria. These morphological differences in susceptible and resistant sorts are inherent, although not absolute, since any variety or strain of alfalfa is made up of a widely diverse lot of individuals. It is for this reason that not a single variety or strain of alfalfa has been found which is completely resistant.

It has been generally found that rapidly growing alfalfas are usually susceptible, whereas slow-growing sorts are relatively resistant. The rate of growth appears to be associated with certain modifications in the structure, which may either favor or inhibit the development of the organism. Water in any form is one of the major factors in modifying the rate of growth. Thus while resistance in alfalfas to wilt is associated with root structure, it is also true that inhibiting or accelerating the rate of growth of either susceptible or resistant sorts will modify the root structure to such an extent that susceptible sorts will become more resistant or resistant alfalfas more susceptible.

The curtailment of organic food reserves in roots of diseased plants bears an important relation to alfalfa wilt, and may be caused by the disturbance of the photosynthetic and food-storage activities. There appears to be no direct evidence in any of our physiological or microchemical studies to show that any internal physiologic function of the plant makes one variety more resistant than another, except insofar as morphological modifications may occur under different environmental conditions.

The Nature of Resistance in Alfalfa to Wilt

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Department of Plant Pathology

Alfalfa wilt was first discovered by Jones (3) in 1924 in southern Wisconsin and northern Illinois. The causal organism, *Aplanobacter insidiosum*, was later described by McCulloch (10). Since that time wilt has been reported from most of the alfalfa-growing sections of the United States and in some states it has been responsible for a rapid decrease in the acreage of alfalfa.

This disease is primarily vascular, although death of the plants may result from bacterial invasion of the parenchymatous and phloem tissues. It can be readily recognized in the field by the conspicuous dwarfing and yellowing of the plants, the bunchy-growth effect produced by the numerous shortened stems, the small, chlorotic, rounded, cupped leaves, and finally by the yellow or brown discoloration in the outer wood of the tap root.

During the past three years a number of investigators (6, 12, 13, 15) have found that certain alfalfas are somewhat resistant to wilt, whereas others are very susceptible. At the Nebraska station the results to date indicate that all common and most variegated alfalfas are very susceptible to wilt. So far only certain alfalfas having their origin in Asia have been found to be relatively resistant. It is apparent that the solution of the alfalfa-wilt problem lies in the development of resistant sorts that are winter-hardy as well as productive, and it is for this reason that a thorough study of the nature of resistance in alfalfas is desirable.

The majority of the investigations dealing with the nature of disease resistance have been carried out with parasitic fungi. Very little has been accomplished with bacteria, except to determine the resistance of crop plants through routine inoculations. The object of this investigation was to determine, at first from anatomical and later from physiological and microchemical studies, why certain alfalfas are resistant, whereas others are susceptible to bacterial wilt.

ANATOMICAL STUDIES

Anatomical studies of alfalfa wilt have been pursued in this laboratory for the past five years.[1] At the beginning these

[1] The senior writer wishes to acknowledge his indebtedness to J. Kramer, former laboratory assistant, and J. H. Jensen, former graduate assistant in the Department of Plant Pathology, Nebraska Agricultural Experiment Station, for their assistance with certain phases of the anatomical studies and to C. E. Rosenquist, of the Department of Botany, University of Nebraska, for the measurements recorded in Table 3.

involved material collected in the field under varying conditions with plants of all ages and stages of infection. As the work progressed the tendency has been to use younger plants inoculated under controlled conditions. During the past two years the studies have been based mostly on plants inoculated at the age of 3 to 12 weeks, because the progress of bacterial invasion could be followed more readily. Most of the comparative studies to be reported later were made with plants inoculated at the age of 3 to 6 weeks.

Likewise, the technique of fixing, sectioning, and staining has been modified from time to time. For the past three years the material has been fixed in formal-acetic-alcohol (5 c.c. neutral formalin, 5 c.c. glacial acetic acid, and 90 c.c. of 50 per cent alcohol), sectioned with a Spencer freezing microtome, and stained with a modified Fleming triple and Gram positive as first employed by Jones (5). The Hucker modification of the Gram stain, as recommended by the Society of American Bacteriologists (17), was substituted for the formula used by Jones.

Although Jones (5) has already made a somewhat detailed study of the progress of the bacteria from entrance to the subsequent death of the plant and although in most instances our studies substantiate his findings, a brief account will be given of the pathological anatomy of alfalfa wilt so that the progress of the disease will be familiar to the readers of the subsequent pages.

PORTALS OF ENTRANCE

So far as it has been possible to determine from our studies and others (5, 7, 12), the bacteria enter the alfalfa plant at any age only through wounds. The kind and the location of the wounds apparently do not make any difference, if the bacteria enter very shortly after they are made. Under field conditions bacteria may enter through the cut ends of stems at the time of mowing and in the early spring through injury wounds of various types, which may expose parenchymatous tissue (4, 5, 8, 12, 14). Occasional observations have been made in cross sections of diseased field plants where entrance seemed to have been effected through rootlets, but no actual proof of entry through wounds on the rootlets can be offered. Also, an occasional slide has suggested the possibility of entrance into the wound formed by a root branch pushing out through the cortex of the main root.

Plants have been infected under controlled conditions through three types of wounds, namely cut stems at any point above the crown, cut roots at any point, and a light super-

ficial scraping of the outer bark of the roots. All wounds, of course, were made under a bacterial suspension. The last-named method of wounding has been generally employed in the inoculation of older plants (13), while the second has been commonly used to inoculate seedlings. The first method was used to good advantage in determining the time of infection through cut stems in order to simulate mowing under field conditions (12).

Of the natural portals of entrance, the root lenticels offer a possible avenue. Studies are now under way to determine whether bacteria can under certain conditions enter the root through the lenticels. In the greenhouse and field, and especially under high soil-moisture conditions, a few uninoculated control plants have become infected and died of wilt. Thus, barring the possibility of root-lenticel infection, it is safe to assume that invasion through wounds of any type or location, both above and below the ground, is the most frequent mode of entry.

INVASION OF THE VASCULAR SYSTEM

When stems are cut in mowing or any above-ground parts are injured, bacteria may enter the vessels if free water is present, for, as pointed out by Jones (7) and repeatedly observed by the writers, the cut stems do not bleed but have an avidity for water. The bacteria are probably carried along into the exposed vessels. After entering they make their way into the vascular system of the plant. Likewise, when the roots are cut off under a bacterial suspension, they enter the functioning vessels along with the water taken up by them. Once the bacteria enter, they develop and are transported into the root, crown, and stem vessels. The rapidity of transport depends on the age of the functioning vessels and the rate of intake and upward movement of water.

The migration of the bacteria after entrance into the intercellular spaces of the cortex parenchyma and until the subsequent invasion of the young outermost vessels has been very well described by Jones (5) as follows: "From the place of entry the bacteria spread through the larger intercellular spaces in all directions. Progress is most rapid along the phloem rays to the interfascicular cambium, which, unlike the fascicular cambium, is almost always composed of comparatively large cells with intercellular spaces. As soon as the bacteria have passed the cambium, they begin to spread tangentially along the middle lamella between the parenchymatous cells of the vascular bundles until they reach the vessels. The outer wall of the vessel appears to offer no resistance to the entry of the bacteria. The bacteria do not

continue to spread indefinitely among the cells of either phloem or xylem from the point of infection." Jones does not state the exact method of entry into the vessels, but infers that they may enter at any point. Repeated observations by the writers show that there is only one portal of entry into the vessels, i. e., through the middle lamella at the pits (Pl. 1, A).

After the bacteria have entered the vessels of several bundles, they multiply rapidly in the pits (Pl. 1, B) and are transported through the anastomosing vessels in all the bundles of corresponding development around the circumference of the roots (Pl. 3, D). They also move down into the roots and up into the crown and into some of the growing stems.

Jones (5) states that "there are three possible routes by which bacteria may pass from vessel to vessel: first, by open passages between vessels; second, by penetration of the thin middle lamella-like walls separating contiguous vessels; and third, by passing through the middle lamellae of walls of parenchymatous cells, separating vessels." The writers have never observed such open communication between vessels as is shown by Jones (5, Fig. 3). They have observed what appeared to be open communications at certain planes in thick sections, but the walls always appeared at other planes. Jones admits that "open communication in a radial section between contiguous vessels has been found in but a single instance." Conclusive evidence that bacteria can pass from one vessel to another through open passages is, then, not at hand.

In discussing the second route by which bacteria may pass from vessel to vessel, Jones (5) states that "it may seem highly probable that they can pass from one vessel to another contiguous vessel by penetrating the lamellae separating their lumina wherever the open spaces between the inner thickenings are opposed. A clear demonstration of this seemingly possible method of distribution has not yet been made." The writers find that this is the usual method by which bacteria pass from one contiguous vessel to another. In other words, the bacteria in the vessels seem to thrive in the pits and are able to penetrate the lamella when two contiguous pits are exactly opposed to each other (Pl. 2, D). It is the only way in which bacteria may pass from one vessel to a contiguous vessel in the same bundle.

The writers' observations agree with Jones' (5) regarding open communicating vessels. In other words, the root vessels are tracheal tubes, rather than tracheids as suggested by LeClerg and Durrell (9), although they state that "Judging by the speed of water flow in a normal alfalfa root it might

be assumed that the water-conducting vessels were long tracheal tubes." The fact that there are few or no cross walls in the vessels permits an uninterrupted movement of the bacteria up and down and around the root in vessels of the same age. It also readily permits movement of the bacteria to the crowns and stems.

The movement of the bacteria in the vessels is somewhat similar to the movement of ink particles. LeClerg and Durrell (9) find that "When India ink is drawn into the roots the larger suspended particles are caught in the pits of the tracheids like drifted sand in a gutter and sometimes pile up in the ends of the cells." A comparison of LeClerg and Durrell's figures (9, Figs. 11 to 14) with those of the writers (Pl. 1, B) shows clearly that the movement of the bacteria in the vessels is primarily mechanical like that of the inert carbon particles.

The experiments performed by Jones (5) and by LeClerg and Durrell (9) with an India ink suspension demonstrate beyond a doubt the open communication in vessels. Further, they also prove that only the functioning vessels take up the ink particles, and in most instances these vessels are located in the bundle nearest the cambium. Similarly the bacteria invade only the functioning vessels when the roots are cut off under a bacterial suspension, and, when the bacteria enter through parenchymatous tissue, they invade the tissue and reach the younger vessels first. An explanation of why the bacteria do not develop in the older, non-functioning vessels will be presented later.

It is evident from many observations of both longitudinal and cross sections of young vessels that in most instances the middle lamellae are common to contiguous vessels. At the point where the pits are deepest, there is not much more than the middle lamella separating the vessels. LeClerg and Durrell (9) find that "In the center of these pits is the middle lamella separating the lumen of adjoining tracheids. The walls of the tracheids are rather thick and the pits are deep and often quite narrow. Observations of the middle lamella reveal a peculiar granular or stippled surface of this part of the wall which gives the appearance of minute pores." They state further that "It is difficult to see, however, how the ink particles pass the middle lamella of the pits. This suggests a porous structure of the lamella."

A detailed study of the pits in functioning vessels has been made because of their apparent importance in the pathogenesis of bacterial wilt. Longitudinal sections have repeatedly disclosed the depth of the pits and the middle lamella practically exposed at the base. The pits themselves show a

somewhat granular and porous composition (Pl. 1, B and C). The same observation has repeatedly been made with infected vessels (Pl. 1, B), namely that the bacteria seem to lodge and fill up the pores in the pits and at times it is difficult to determine whether or not the bacteria have actually passed through the middle lamella.

The fact that the pits are not of a homogeneous nature, but rather of a porous heterogeneous structure, is of importance since the bacteria lodging in the pores may find their way through the matrix of pores and the middle lamella more easily than if the pits were composed of a uniform matrix. Thus it is apparent why passage of the bacteria into a functioning vessel and their exit (Pl. 1, D) into a contiguous vessel or out into the parenchymatous tissue is rather easily accomplished.

BACTERIA IN THE PARENCHYMATOUS TISSUE

The bacteria push out through the intercellular spaces and either invade the parenchymatous tissue (Pl. 3, A, B, and C), following outward along the medullary ray cells into the phloem, or they may enter other functioning vessels farther out in the radial bundle. As a rule the entrance and invasion of the ray cells and phloem are dependent on the rapidity of plant growth and the environment. The rapidity of invasion of the bacteria in different varieties of alfalfa and the influence of age of the plant, maturity of the cells, and environmental reaction will be taken up somewhat later.

Up to this point the discussion has been confined to root tissues. Conditions in the stems are somewhat different since they are cut off several times during the summer for hay. Bacteria have always been found in the stems and leaves of plants having bacteria in the root and crown tissues. The extent of the bacteria in stems is related to the number of functioning vessels infected in the root and crown. Thus not all stems and leaves of an infected plant contain bacteria, because the xylem elements in the stem connected with non-infected root vessels would remain healthy. Even in a plant showing decided dwarfing and other characteristic symptoms of wilt, all stems would not necessarily contain bacteria. In the stem xylem the bacteria are usually found in some of the innermost vessels and in the pith (Pl. 4, A and B). Only occasionally have bacteria been found in the outer xylem elements and in no instance have bacteria been seen in the phloem region of the stems. At no time did the writers find bacteria as plentiful in the stems and leaves as in the roots, although no diseased plant has yet been found that did not have some bacteria present in the stem tissues.

For the past five years a number of attempts have been made to determine whether bacterial wilt is seed borne. To date all such experiments have proved negative. Jones (5) found bacteria at the base of the pedicel bearing a seed pod and he observed a few bacteria in a stained section in the base of a seed pod, but he found none near the hilum of the first seed in the pod. He also states that "In no case were the bacteria found abundantly in the upper part of the seed plants." The writers agree with Jones that if seed infection does occur, it is only in rare instances.

RELATION OF AGE OF THE PLANT AND CELLS TO BACTERIAL INVASION

Three-weeks-old seedlings have been readily infected by cutting the roots under a bacterial suspension. A section of the roots of seedlings at this age shows the beginning of the secondary xylem elements, although the primary xylem is still present (Pl. 5, A and B). In some instances bacteria have been found in the triarch bundle of the primary xylem and in the vessels of the secondary xylem. Sections of plants three months old and older show only the secondary xylem. The bacteria do not enter all the vessels but only those nearest the cambium at the time of infection (Pl. 6, A and B). Perhaps the bacteria enter some of the older vessels, but if they do they apparently fail to develop, since no evidence of their presence can be seen even a few weeks after inoculation. With plants one year old or older the bacteria enter only those vessels on the outer rim nearest the cambium. Here again it is possible that the bacteria enter some of the older vessels but they are not evident when the roots are sectioned. Thus, while plants of any age may be successfully infected with wilt under controlled conditions, the organism enters only the young, actively functioning vessels.

There may be two explanations for this, namely: that the bacteria develop and multiply only in the young vessels which carry food materials and especially soluble sugars, or they develop and multiply in the pits of the young vessels at the expense of the soluble pectin present. Whatever may be the reason, it is evident that they are able to grow only in those vessels nearest the cambium. Thus, while the age of the plant is not of any importance so far as successful infection under either natural or artificial conditions is concerned, the age of the vessels is the limiting factor in the successful invasion of the plant. Once the bacteria make an exit from infected vessels, the parenchymatous tissue surrounding them is uusally susceptible to bacterial invasion. If environmental conditions are favorable the bacteria reach the cambium and phloem, causing a disorganization of these tissues and finally the

death of the plant. The fact that the bacteria never move inward but always outward is due solely to the maturity of the cellular elements. Sections of plants inoculated at various ages, from three weeks onward, indicate that the maturation of the vessel elements is a rapid process, since individual vessels of one month old or older are not invaded by the bacteria (Pl. 6, A and B). Apparently it is primarily a food relation rather than strictly a matter of water conduction of the vessels.

Under field conditions in Nebraska spring-seeded plants have been found infected in the fall (12). Several instances have been found where plants seeded either in the spring or fall on wilt-infested soil have been found infected in the spring. Jones (5) reports one instance of wilting of seedlings in their first year. At the other extreme, plants have been found recently infected in a forty-year-old field. Peltier and Jensen (12) find that the time of infection of new plantings varies considerably with the previous crops, environmental conditions, and the proximity of infected fields. Usually, under optimum conditions for infection, wilt can be found in two-year-old fields, while stand reduction may be evident the third or fourth years. So far as the age of the plants is concerned, plants may be artificially inoculated or naturally infected at any age. The life of infected plants varies from a few weeks to several years, and at times plants may completely recover. Seedlings usually succumb in a shorter time than older plants.

MORPHOLOGICAL STUDIES OF SUSCEPTIBLE AND RESISTANT ALFALFAS

For the most part the pathogenesis of bacterial wilt has been studied in susceptible varieties, particularly Grimm. Heretofore no attempt has been made to compare the anatomy of susceptible and resistant alfalfas. In the following paragraphs a comparative anatomical study of Grimm, Turkestan, and other alfalfas will be reported. In most instances plants of the same age were inoculated and grown under comparable conditions. The fixed material was always taken at about the same point, so that the sections represent comparable tissues. It must be borne in mind, however, that each alfalfa represents a widely diverse group of individuals, and for this reason exceptions may be found in any of the alfalfas under discussion.

One of the most striking differences observed in cross sections of susceptible and resistant alfalfas is the relative diameter and construction of the xylem elements. In Turkestan alfalfa the vessels are not only strikingly smaller but also, in most instances, they appear angular with heavy wall

thickenings, whereas in the susceptible sorts the vessels are more rounded, much larger in diameter, and have secondary and tertiary walls of less thickness. Growth of the vessels is apparently slower, as indicated by their size, and their maturity is more rapid because of the heavier walls in Turkestan than in Arizona (Pl. 7, A and B). In other words, the time from the inception of the vessel to maturity is much shorter in Turkestan.

The second most striking difference can be observed in longitudinal sections. In Grimm the vessels are composed of rather long cells in which the cross septations have disappeared to form long open communications (Pl. 2, A). Only occasional vestiges of the septations can be seen. In Turkestan the cells making up the vessels are shorter and larger vestiges of the cross walls are still present (Pl. 2, B). The bacteria encounter few if any obstructions in their passage through the vascular system of Grimm and other susceptible alfalfas, whereas in the smaller vessels of Turkestan they do meet with many obstructions in the form of partial dams (Pl. 2, C) back of which the bacteria have a tendency to pile up, thus interfering with rapid passage through the vascular system of the plant. For the most part resistant alfalfas have angular vessels of smaller diameter, which apparently mature more rapidly and have many partial obstructions which interfere with the rapid transport of the bacteria, whereas susceptible sorts have rounded vessels of larger diameters, and the obstructions in the vessels are absent for the most part.

The number and arrangement of the vessels appear to affect the degree of resistance to wilt. A radial bundle of vessels in Arizona consists of a loose grouping of single vessels, with only an occasional sheath of vessels, and with few breaks between them, filled mostly with wood parenchyma cells and a few wood fibers (Pl. 7, B). Generally in Turkestan the vessels are laid out in groups rather than singly in the bundle. The sheaths of several vessels are surrounded by wood fibers and some wood parenchyma cells (Pl. 7, A). The radial spread of the bacteria from one contiguous vessel to another would be much more rapid in those roots in which the bundles are loosely arranged in single rows, with only a few breaks, than where the vessels are arranged in groups and more or less surrounded by wood fibers. Further, it has been determined that the bacteria cannot advance through the wood fibers, since they are composed of insoluble lignin.

The parenchymatous tissue (wood rays) are often directly in contact with the vessels in Arizona (Pl. 7, B). In resistant sorts the wood rays are seldom in direct contact with the vessels, owing to the greater number of wood fibers sur-

rounding them (Pl. 7, A). Further, the walls of the parenchyma cells of the medullary rays of the resistant sorts appear to be relatively heavier and they have smaller intercellular spaces (Pl. 7, A). In the roots of Arizona the parenchymatous tissue appears to be arranged less compactly, and the size of the intercellular spaces is larger (Pl. 7, B).

Outside of the greater number of wood fibers present separating the vessels from the medullary rays, which in many cases extend beyond the cambium, no morphological difference can be found in the cambium and phloem regions of susceptible and resistant sorts.

In Turkestan and other resistant alfalfas the arrangement of the vessels in the bundles greatly inhibits the movement of the bacteria from one group of vessels to another, since they are not so often in direct contact outwardly in a radial direction. The bacteria may pass from one contiguous vessel to another when they lie in a group, but the fact that they are surrounded for the most part by wood fibers through which the bacteria cannot pass to reach the parenchymatous tissues greatly inhibits their spread to the younger developing vessels. Many cross sections of resistant alfalfa roots have been examined in which it is apparent that, although entrance of the bacteria was easily effected, the bacteria were limited to one or more groups of vessels. If the bacteria fail to invade other vessels in the same bundle or the medullary rays, the plant remains healthy.

In Grimm and other susceptible sorts the bacteria progress from one vessel to another more readily, and, where breaks occur, can in most instances invade the wood parenchymatous tissue directly and either enter other young developing vessels or continue between the cells of the medullary rays to the cambium and phloem. In most instances the type of vessels, their arrangement in the radial bundle, and the amount of wood fibers surrounding them distinguish the root sections of susceptible and resistant sorts.

This conclusion has been based on an anatomical study of sections from a large number of susceptible alfalfas and the few resistant sorts that have been obtained up to the present (Pl. 8). These plants were all inoculated at the same age and grown under comparable conditions in the greenhouse. In most instances the root structure can be correlated with the amount of growth produced by these alfalfas under field conditions. Hay yields which reflect the rate of growth show for the most part that the high-yielding alfalfas are of the variegated type, such as Grimm or some of the selected Common types produced in a number of states, while some Turkestans and similar types produce in many instances unsatisfactory yields in comparison.

In general it has been found (6, 12, 13, 15) that high-yielding alfalfas are usually quite susceptible to wilt, whereas the lower-yielding sorts, like Turkestan, are relatively resistant. Rapid growth in an alfalfa is reflected in the structure of the root (1) by the length of the cells making up the vessels; (2) by their large diameter, shape, arrangement, and rate of maturity as evidenced by the delay in producing secondary and tertiary thickenings; and (3) by a minimum of wood fibers surrounding the vessels, and an abundance of parenchymatous tissue with large, thin-walled cells, loosely grouped, allowing for larger intercellular spaces. This latter type of root structure allows for a rapid and usually fatal bacterial invasion (Pl. 8, A and B). Slower-growing alfalfas on the other hand have (1) vessels composed of short cells, the septations of which have not been entirely dissolved; (2) vessels of small diameter, angular shape, with secondary and tertiary cell wall thickenings laid down early, denoting their rapid maturation and their arrangement in sheaths usually surrounded by fibers; and (3) a smaller amount of small thick-walled parenchyma ray cells compactly arranged with few open intercellular spaces (Pl. 8, C and D).

TABLE 1.—*The percentages of healthy, diseased, and dead plants at one and five months after inoculation of three-weeks-old seedlings of four alfalfas.*[1]

Kind of alfalfa	F. C. I. or F. P. I. accession number [2]	After one month			After five months		
		Healthy	Diseased	Dead	Healthy	Diseased	Dead
		P. ct.	*P. ct.*	*P. ct.*	*P. ct.*	*P. ct.*	*P. ct.*
Arizona	15837	66	34	0	7	21	72
Grimm	15936	46	54	0	0	21	79
Provence	34886	95	5	0	82	0	18
Turkestan	15754	92	8	0	84	8	8

[1] Thirty plants of each alfalfa were inoculated by severing the ends of the seedlings under a bacterial suspension.

[2] The letters F. C. I. and F. P. I. indicate, respectively, the accession numbers of the Division of Forage Crops and Diseases and the Division of Foreign Plant Introduction, Bureau of Plant Industry, U. S. Department of Agriculture.

In some alfalfas both types of plants can be found. Ladak is a rather good example, as it is a very heterogeneous variety. Numerous infection tests indicate that roughly half the plants are susceptible to wilt and the remainder relatively resistant. Cross sections of the roots revealed the typical differences in structure enumerated above. The same thing may be said of a large number of alfalfas which show promise as resistant sorts (Pl. 9). Up to the present in highly susceptible sorts, however, no resistant types have been found.

Plants of two susceptible and two resistant alfalfas were inoculated at the age of three weeks. The method of root severance under a bacterial suspension was followed. The seedlings were then planted in flats and grown in the greenhouse under optimum conditions. At the end of one month visible top symptoms were noted and detailed observations of both top and root symptoms were made when the experiment was discontinued, five months after inoculation. The results are presented in Table 1. It will be noted that 34 and 54 per cent of the two susceptible alfalfas, respectively, showed typical wilt symptoms of varying degrees of severity one month after inoculation. Only an occasional plant of the resistant sort had slight symptoms of wilt. Even after a five-months period 82 and 84 per cent, respectively, remained healthy, indicating that though entrance of the bacteria may have been effected, in only a few plants were they able to multiply and invade the root tissues to produce typical symptoms or death of the plants (Pl. 5, B). In the susceptible sorts, however, practically all the plants were either badly diseased or dead (Pl. 5, A). There can be no question but that when seedlings of resistant and susceptible sorts are inoculated and grown under comparable conditions, a high mortality is produced among the susceptible ones and the reverse in the resistant sorts.

A supplementary lot of these same alfalfas was inoculated and grown under similar conditions and a few plants of each sort were sectioned at intervals during the five-months period. The first plants, sectioned two weeks after inoculation, did not show any visible top symptoms. Only one infected vessel of Turkestan was found in the sections, while 3 to 6 vessels of Grimm and Arizona were heavily invaded by the bacteria. At the end of three weeks 1 to 6 vessels of Turkestan contained bacteria and a considerable number of vessels in the Grimm and Arizona roots were filled with bacteria, although at the time the bacteria were still confined to the vessels, at least in the sections examined.

The relative progress of invasion in an Arizona and a Turkestan plant, both of which showed definite top symptoms, six weeks after inoculation is shown in Plate 6, A and B, while the extent of invasion in a Grimm and a susceptible Turkestan plant at the end of a two-months period is shown in Plate 5, A and B. It can be readily seen that the bacteria have progressed outwardly in the radial bundles to an extent of approximately half the innermost vessels, in the Grimm root, in contrast to the Turkestan root grown under identical conditions which has only a few vessels in most of the radial bundles around approximately the same circumference in-

vaded by the bacteria. The various cellular elements, which inhibit the invasion of the tissues by the bacteria, can be easily discerned.

RELATION OF WATER TO ROOT STRUCTURE IN SUSCEPTIBLE AND RESISTANT ALFALFAS[1]

All investigators are agreed that alfalfa wilt is more prevalent where abundant water in the form of precipitation, subsoil moisture, or irrigation is available. Peltier and Jensen (12) have classified the prevalence and seriousness of wilt on the basis of available water in various alfalfa-growing sections of Nebraska. Of course the amount of water available to the alfalfa plant is reflected directly in the rate of growth and yield of hay, other conditions being favorable for growth, and while free water is essential for the entrance and spread of the bacteria, its relation to plant growth is of prime importance.

In order to determine the influence of soil moisture upon the development of wilt, an experiment was undertaken with seedlings of four alfalfas. Six-weeks-old seedlings of two susceptible and two resistant sorts were inoculated by severing the ends of the roots under a bacterial suspension and transplanted into flats and grown in the greenhouse under optimum conditions. All flats were watered equally for a two-weeks period until the plants had established themselves in the soil. Thereafter the plants were grown under conditions of low, medium, and high soil moistures, respectively. Notes were taken from time to time on the development of the plants and appearance of the top symptoms. Root specimens were also fixed from time to time for microscopic studies of stained sections. The results of the experiment are presented in Table 2.

At the high soil moistures, 100 per cent of the plants of the two susceptible sorts showed severe symptoms or were dead four and one-half months after inoculation. A somewhat similar situation occurred in the medium-soil-moisture lot. With the low soil moisture, however, 60 per cent of the plants remained healthy. Of the two resistant sorts, 90 per cent were healthy with the low soil moisture, approximately 80 per cent with the medium, and 70 and 76 per cent, respectively, for Provence and Turkestan with the high-moisture set.

Fewer plants of the resistant sorts developed symptoms or died in the high-soil-moisture set than of the susceptible sorts

[1] Since the completion of this manuscript for publication, there appeared Bulletin 378, of the Ill. Agr. Exp. Sta., by B. Koehler and F. R. Jones, entitled, "Alfalfa wilt as influenced by soil temperature and soil moisture." In this bulletin the authors point out the effect of soil temperature and soil moisture on root structure and also the movement of wilt bacteria in the host tissue. Their results agree, in the main, with those presented on the same subjects here.

TABLE 2.—*The percentages of healthy, diseased and dead seedlings of four alfalfas grown under low, medium, and high soil moisture for a period of four and one-half months after inoculation at the age of six weeks.*[1]

Kind of alfalfa	F. C. I. or F. P. I. accession number	Healthy	Diseased	Dead
		Per cent	*Per cent*	*Per cent*
LOW SOIL MOISTURE				
Arizona	15837	60	30	10
Grimm	15936	60	30	10
Provence	34886	90	0	10
Turkestan	15754	90	0	10
MEDIUM SOIL MOISTURE				
Arizona	15837	10	90	0
Grimm	15936	0	80	20
Provence	34886	82	9	9
Turkestan	15754	80	20	0
HIGH SOIL MOISTURE				
Arizona	15837	0	70	30
Grimm	15936	0	43	57
Provence	34886	70	0	30
Turkestan	15754	76	12	12

[1] Ten seedlings of each alfalfa under each condition were inoculated by severing the ends of the roots under a bacterial suspension.

with the low soil moisture. High soil moistures were conducive to the development of wilt in the resistant sorts because of the modifications in the root structures of these plants, and they approximated the root structures of the susceptible sorts grown at low soil moistures. The relation of water to plant growth must be considered a primary, but indirect, factor in the relative susceptibility of alfalfas.

A photomicrograph of a stained cross section of the root below the crown of a Turkestan plant grown under low soil-moisture conditions is shown in Plate 10, A, in contrast to one (Pl. 10, B) grown with high soil moisture. Only a few vessels at the center of the root have been invaded by bacteria. Careful examination of the sections reveals that the vessels of the radial bundle are relatively small in diameter, have comparatively heavy angular walls, and are frequently separated or surrounded by conspicuous sheaths of wood fibers. Evidently the bacteria were able to enter and develop in a few vessels but were not able to effect an exit from them, either to invade the parenchymatous tissue or other young vessels, and so the plant continued to grow and remained healthy. In a similar section of a root grown under high

soil-moisture conditions, bacteria are present in a considerable number of vessels in the radial bundles. Here the vessels are relatively large in diameter and more or less rounded and thinner-walled, while the wood ray cells and cortex cells do not have as thick walls as in the foregoing photomicrograph. There is also a looser organization of the medullary rays and possibly fewer wood fibers surrounding the vessels.

Sections of Grimm roots grown under low soil-moisture conditions appear to have a morphological structure somewhat similar to that of sections of Turkestan grown in high soil moisture, namely smaller vessels (angular and thick-walled), more compact parenchymatous tissues, and possibly a more compact arrangement of wood fibers. A photomicrograph of a root section of a diseased Grimm plant grown under a medium soil moisture is presented in Plate 11, A, in contrast to a control plant (Pl. 11, B) grown under similar conditions. These plants show very clearly the effect of the disease on the various component parts of the root, namely the number of infected vessels, the disorganization of the tissues, and the rapid advance of the bacteria, which resulted in the death of the plant.

From the results of this experiment and in the light of the pathological anatomy studies of sections of plants grown under varying soil moisture conditions, we can conclude that, while there are inherent morphological differences between resistant and susceptible alfalfas due primarily to their habits of growth, these morphological differences may be so modified that a Grimm plant may be relatively resistant under low-soil-moisture conditions, whereas under a high-soil-moisture condition, Turkestan plants may become somewhat more susceptible. Water, therefore, is an important factor in modifying the structure of alfalfa roots and its influence is directly reflected in the growth rate of the plant.

Thus under dry or wet conditions in Nebraska, Turkestan will maintain a stand for a longer period than Grimm, although Turkestan grown under irrigation will not maintain a stand as long as other fields of Turkestan grown under dry-land conditions. Similarly the average life of a Grimm field under irrigation is only two to four years, while under dry-land conditions stands may be maintained for two or three times as long a period.

The influence of water on the structure of alfalfa roots up to this point has been based solely on observed differences in stained sections. In order that more exact quantitative data might be obtained the following experiment was undertaken. Plants of Grimm and Turkestan from seedings made the preceding spring were brought in from the field in late February.

These plants were still dormant. After careful selection for uniformity was made, they were planted in cans held at three different moisture contents and placed in soil temperature tanks. To obtain a low, optimum, and high soil moisture, 35, 55, and 70 per cent soil-moisture equivalents were employed. It was determined that 15, 23.6, and 30 per cent actual soil moisture were represented. All three soil moistures were run at soil temperatures of 14°, 18°, 22°, 26°, and 30° C. By weighing the cans each day and adding the water as required, the soil moistures were maintained for the period of the experiment. The plants were allowed to grow under these conditions for three months, at which time the tops were cut off and immediately weighed, while a portion of the roots just below the crown was fixed and later stained sections were prepared for a morphological comparison. In making this comparison, only the new growth formed while the plants were in the tanks was considered.

A fixed area was measured under high power, which included part of a radial bundle containing two or more vessels. These vessels were placed in the center of the field and all tissues within the fixed area measured. Six areas were measured, beginning at the outside margin of the section and systematically moving to every third bundle, toward the inside ring of the new growth. The area occupied by the vessels was obtained by measuring the diameter of all the vessels. Wherever possible the area of all wood parenchyma was measured in total, but where it was scattered, diameters of the individual cells were determined. The remaining area was taken as that occupied by wood fibers. While the data are not absolutely exact, they represent a very close approximation of the component elements of the fixed area measured. It must be borne in mind, however, that at the various temperatures and moistures the area of new growth of the plants varied. At low temperatures and with low soil moisture the amount of new growth was less than at higher temperatures with high soil moistures. The data presented in Table 3 for the Grimm plants at two soil moistures and five temperatures give the approximate components of equal areas of new growth produced under the varied conditions of soil temperatures and moisture. It is quite apparent that soil moisture has a profound effect on growth, as well as temperature, since the average top growth of all plants grown at low soil moisture at all temperatures was only 17.2 grams per plant, while at high soil moistures the weight of the tops was 45.2 grams per plant, almost three times as much. In the main the results show that under low soil-moisture conditions the diameter of the vessels is much smaller since only approx-

imately 23 per cent of the area measured is taken up by the vessels, whereas 40 per cent of the same area is occupied by the vessels in plants grown under high soil-moisture conditions. There is not any difference in the area taken up by the wood parenchyma under these two conditions. The difference in the area occupied by wood fibers, however, is almost the same as that of the vessels, but inversely so. Normally under low soil-moisture conditions 43.6 per cent of the area consists of wood fiber and only 27.8 per cent of the area is so occupied in the high-soil-moisture series. In other words Grimm plants grown under low soil-moisture conditions tend to produce vessels of small diameters with more wood fibers, conditions which control or inhibit the disease, whereas under high soil-moisture conditions vessels of larger diameter are produced with fewer wood fibers, a condition which favors the rapid invasion of the bacteria.

TABLE 3.—*The influence of soil moisture at five soil temperatures on the root structure of plants of Grimm alfalfa*

Soil temperature	Low soil moisture (15 per cent)				High soil moisture (30 per cent)			
	Area of vessels	Area of wood fibers	Area of wood parenchyma	Av. wet wt. of tops	Area of vessels	Area of wood fibers	Area of wood parenchyma	Av. wet wt. of tops
°C	*P. ct.*	*P. ct.*	*P. ct.*	*Gr.*	*P. ct.*	*P. ct.*	*P. ct.*	*Gr.*
30	24.8	45.7	29.5	27	32.2	41.1	26.7	69
26	28.0	48.4	23.6	16	47.3	27.7	25.0	36
22	23.1	34.3	42.6	12	38.8	15.9	45.3	45
18	19.5	37.6	42.9	18	36.8	13.1	50.1	56
14	21.4	51.8	26.8	13	46.9	41.3	11.8	20

PHYSIOLOGICAL STUDIES

THE ORGANISM

Probably the most characteristic features of *Aplanobacter insidiosum* are its extremely slow growth, particularly in liquid media and on most solid media, its avidity for sugars, its production of a pigment of various shades, its apparent variability in growth and color on different lots of similar media, and its loss of virulence in old cultures. The bacteria are Gram positive, which makes them easy to identify. The slow growth of the organism in culture makes it rather difficult to isolate and to study physiologically, since one is never quite sure from the small numbers of bacteria present whether positive or negative results are obtained.

At this time only those studies concerning the ability of the organism to utilize starch, sugars, and pectins for growth will be considered. McCulloch (7) reported that she obtained

a moderate amount of diastatic action on starch suspended in beef agar and on potato cylinders. In our experiments soluble starch agar, together with potato cylinders and thin discs, were employed to test diastatic action. The formula for soluble starch agar and the procedure as employed by Peltier (11) were utilized. The results from a number of tests showed that the bacteria grew very poorly on the soluble starch agar and only a small amount of growth was recorded at the end of two weeks. When the plates were flooded with potassium iodid and iodin only a small clear zone immediately surrounding the colonies was obtained. Similarly on potato cylinders and discs only a small amount of growth was recorded and only a slight amount of diastatic activity was found at the end of two weeks.

In order to determine whether the bacteria could utilize pectin the following experiments were undertaken. Dry pectin extract from "Certo" as prepared by Wolf (18) was obtained and about one per cent by weight of powdered pectin was added to Pfeffer's solution and agar. The procedure used by Wolf (18) in adjusting and maintaining the hydrogen-ion concentration of the pectin agar was followed. At the end of ten days the bacteria had made almost as rapid a growth on this pectin agar as on potato-lactose agar and a much greater growth than on soluble starch agar. Likewise, only a meager growth was made in Pfeffer's solution plus one per cent lactose during the same interval. From these tests we can infer that the rapid growth of the bacteria in pectin agar was made at the expense of the soluble pectin. Further, in testing the hydrogen-ion concentration of pectin agar at the conclusion of the experiment it was found to have changed from a pH of 6.5 to 6.1. This change in acidity according to Wolf (18) is indicative of pectin fermentation.

As a supplementary experiment thin discs of mature carrots, cut under asceptic conditions, were added to inoculated flasks of Pfeffer's solution plus one per cent of lactose. At the end of two months no maceration of the carrot discs was found, nor with ruthenium red could any disappearance of pectin be detected. Evidently while the organism can utilize pectins as extracted from "Certo", it can not attack the pectins found in mature carrots, at least under the conditions of this experiment.

The addition of sugars, particularly lactose, to any liquid or solid media will always enhance the growth of the organism. The addition of one to two per cent by weight of lactose to most media seems to produce optimum growth. McCulloch (7) has found that the bacteria can ferment with slight indication of acid formation, dextrose, lactose, sucrose, and galac-

tose when one per cent of the sugars is added to beef agar. She reports abundant growth on all solutions. With Pfeffer's solution plus one per cent by weight of the above sugars together with arabinose, the bacteria grew fairly well in all cultures and at the end of a ten-day period the solutions were all slightly more acid, showing that fermentation was accompanied by some slight acid production. Thus *Aplanobacter insidiosum* has only a weak diastatic activity, has an avidity for sugars, particularly lactose, and grows readily on agar containing the soluble pectins extracted from "Certo".

THE HOST

The two characteristics of the host plant which are significant here are its high water requirement, its avidity for water, and its ability to use the tap root as a storage organ for organic food reserves. In Nebraska in the various alfalfa-growing sections, water is apparently the limiting factor for growth and yields are directly proportional to the amount of water available. Further, the water requirement of alfalfa is extremely high. Shantz and Piemeisel (16) have shown from their studies at Akron, Colorado, that the water requirement of alfalfa over a seven-year period averaged 859, or if the water requirement of Proso millet is considered as 1.00 the water requirement of corn would be 1.31, wheat 2.09, and alfalfa 2.81. Thus water is very essential to growth and indirectly favors the rapid development of wilt.

Graber and his associates (2) have within recent years pointed out the great importance of organic food reserves in the normal growth and metabolism of alfalfa. They have in the main substantiated the statement "That new top growths, especially in the early stages, are initiated and developed largely at the expense of previously accumulated organic reserves; that the roots of alfalfa are not only organs of absorption and translocation, but are organs of storage for such reserves; that such storage occurs principally during the maturation of top growth; that these organic reserves are essential to normal top and root development; that their quantity, quality, and availability sharply limit the amount of both top and root growth which will occur; and that progressive exhaustion of such reserves by early, frequent and complete removals of the top growth results ultimately in the death of the plant, regardless of the most favorable climatic and soil environment." They have also observed that the amount of winter injury in alfalfa plants may be correlated with the time and number of the cuttings during the summer.

THE DISEASE

Photosynthetic activities of diseased plants.—Apparently there is a disturbance of normal chlorophyll activity in diseased leaves, since in cupped leaves the chlorophyll cannot be completely extracted with either boiling alcohol or chloralhydrate. The remaining chlorophyll usually appears to be segregated in irregular patches between the larger veins near the base of the leaf. Sections through diseased leaves reveal, in many instances, plasmolized cells. Although it has been impossible to find bacteria in all cupped leaves and dwarfed stems, they have been found within the midrib and larger veins of some leaves but never out in the phloem region. It is difficult to believe from the number of bacteria usually present in diseased leaves that they have a direct effect on the chlorophyll and on cellular activity.

Boiling alcohol and chloralhydrate were used as clearing agents, together with a potassium iodid and iodin solution in a series of tests for the presence of starch in dwarfed stems and cupped, chlorotic leaves taken from plants grown in the greenhouse and showing various stages of the disease. Starch was found in these leaves at all hours of the day and night, the amount depending on the extent of dwarfing and cupping. These starch accumulations were particularly pronounced at the tip of the leaves, in the petioles, and at times at the upper nodes of the stems. The amount varied directly with the severity of the top symptoms. It is readily apparent that the translocation of starch is interfered with in some manner. The exact cause of this lack of transport of starch from diseased leaves has not as yet been determined.

Organic Food Reserves in Diseased Plants.—Root dwarfing in diseased plants is almost as conspicuous as the dwarfing of the top growth, although this symptom has not been pointed out heretofore. The severity of root dwarfing can be readily seen when inoculated and control plants of the same age are compared. Photomicrographs of two such Grimm plants are presented in Plate 11. In these sections the amount of root invasion can be readily seen as well as the contrast in the diameter between the diseased and the healthy plant. A similar situation is shown in Plate 10, which shows Turkestan plants of the same age, inoculated and grown in low and high soil moistures. Even though the diseased plant was grown under conditions more favorable for the enlargement of the root diameter, it was much smaller than the one grown in a low soil moisture.

The most conspicuous symptom in the storage cells of the root of infected plants is the absence of starch. The contrast between diseased and control plants (Pl. 11) is very evident. In the control Grimm plant the cells of the wood parenchyma

and medullary ray cells were gorged with starch, while in the diseased plant starch was almost completely absent in all the cells of the medullary rays and wood parenchyma. This same situation is shown in Plate 10 with Turkestan plants.

Plate 12 shows photomicrographs of two infected vessels and two healthy vessels in approximately the same circumference but in different bundles of the same section. In the wood parenchyma cells surrounding the invaded vessels, starch is almost completely absent. In similar cells around the healthy vessels it is abundant. In other sections it has been observed that the wood parenchyma cells surrounding healthy bundles are always filled with starch and that usually starch is absent in cells surrounding bundles having diseased vessels. Therefore it is very evident that the disease not only seriously impairs normal chlorophyll activity in the leaves and disorganizes normal translocation processes, but also prevents the storage of organic food reserves in the roots, which results in the dwarfing of both top and root growth to a point where the plant eventually succumbs.

Under field conditions diseased plants go into dormancy in the fall with varying amounts of root reserves, depending on the severity of the bacterial invasion. Those plants which are extremely low in reserves fail to survive the winter. Others, barely able to survive, are either so injured that they do not survive long or they may start a meager new growth at the expense of the remaining reserve, but, failing to establish sufficient top growth to carry on normally, finally die. Still others have sufficient reserve to make sufficient new growth to develop a fairly good top growth. At the time of the first cutting, however, either little or no new reserve has been accumulated, or the disease has progressed to a point where reserve accumulation is inhibited so that after a meager new top growth is produced, they succumb. Usually for a short period after the first cutting, the most typical symptoms of wilt can be found in infested fields in Nebraska.

Several years' observation of a number of outlying field plots has shown that when a field becomes infested the stands of non-hardy and susceptible sorts decrease most rapidly, followed by the hardy, variegated, susceptible sorts such as Grimm. Stands of mid-hardy but somewhat less susceptible sorts persist for a longer period than Grimm, while the decrease in stands of the hardy but resistant sorts is slow.

It has also been noted in infested fields that the stands decreased much more rapidly when cut four rather than three times a season. The rate of stand decreases in infested fields of Grimm, Common, and Turkestan is chiefly due to the slower progress of wilt once it enters the last two alfalfas.

Apparently the curtailment of organic food reserves in roots of diseased plants is as important a factor as the actual disorganization of the root tissues by the bacteria or, as has been suggested by some investigators (5, 9), to the plugging of the water-conducting vessels. Intensive studies are being pursued to determine the factors or agencies involved in the disturbance of the chlorophyll, translocation, and storage activities of diseased alfalfa plants. There is no direct evidence in any of our physiological studies that any internal physiologic function of the plants makes one variety more resistant than another, except that morphological modifications may take place under different environmental conditions.

MICROCHEMICAL STUDIES

It has been shown that the bacteria develop and multiply only in the young functioning vessels after entrance is effected. As to what materials they utilize for energy and growth, three possibilities are apparent. The young vessels contain organic materials favorable for their growth, or they utilize the soluble pectin in the vessel walls, or else the bacteria are able to draw on the food materials stored in the wood parenchyma or medullary ray cells surrounding the invaded vessels.

Dixon and Ball (1) believe that in the translocation process a solution of organic nutrients, having passed from the synthesizing cells of the leaf because of increased permeability, will be drawn down through the xylem tubes by the water columns in tension and will be absorbed at lower levels by the living cells and distributed laterally throughout the stem and root. *Aplanobacter insidiosum* makes an extremely meager growth in synethic liquid cultures, but a more favorable growth occurs when sugars, particularly lactose, are added to these cultures. If we are to adopt the hypothesis that the bacteria develop at the expense of the vessel fluid, we must assume that organic nutrients are found in the young vessels.

A very good growth of the bacteria is also obtained on pectin agar, and in young, recently infected vessels they seem to multiply rapidly in the pits rather than in the lumen of the vessel. Microchemical tests of such vessels with ruthenium red (aqueous solution 1:5,000) indicate that pectin is abundant in the middle lamella and in part of the cell walls at the pits. This same test applied to older and more mature vessels shows that even the middle lamella responds weakly, whereas when tests with pluroglucin-Hcl are made a positive reaction for lignification is obtained. As the bacteria increase and fill the lumina, an insoluble gum is produced which frequently fills the vessels. Microchemical tests with pluroglucin-

Hcl of this gum shows that it has a lignin composition. It can be argued that the bacteria use the soluble pectins present in the middle lamella and vessel walls as a source for energy and growth, and that the insoluble gum, so commonly present in infected vessels, is a residue of the bacterial action on the soluble pectins.

The third possibility is that the bacteria in the vessels are able to draw upon the organic nutrients present in the wood parenchyma and medullary ray cells surrounding the vessels, since it has been found that starch is absent from these cells in the neighborhood of the invaded vessels. How this might be accomplished is not known, since we know that the bacteria have only a slight diastatic activity.

Further studies are in progress to determine, if possible, the actual source of food for the development of the bacteria in the young functioning vessels. It is apparent, however, that whatever this source may be it is plentiful over a longer period in susceptible sorts than in resistant alfalfas, since the vessel walls of the resistant sorts mature and become lignified more quickly than those of susceptible alfalfas.

The maturity of the vessels in a radial bundle can be roughly determined from the intensity with which the walls take up safranin and by the intensity of their reaction with pluroglucin-Hcl and ruthenium red. The wood fibers react positively to the lignification test with pluroglucin-Hcl and in no instance have bacteria been found invading their intercellular spaces. The wood fibers therefore act as a barrier against bacterial invasion. Fibers interspersed between and around the vessels in a radial bundle are very effective barriers against the bacterial invasion of the new developing vessels and the wood parenchyma and medullary rays.

No indication of lignification of the cell walls was observed in any of the parenchymatous tissue in the stele and cortex. The middle lamellae of the parenchyma cells both in the stele and cortex react readily with ruthenium red, indicating that pectin or pectin-like substances are present. Positive tests were also obtained for cellulose in the secondary and tertiary walls of these cells.

Apparently the middle lamella is acted on in advance of the bacteria, since a deep staining reaction is obtained (Pl. 4, C) with safranin, usually in the intercellular spaces of the wood parenchyma and ray cells in the vicinity of plugged vessels and also when bacteria are present in the medullary rays in the parenchyma cells of the cortex. A staining reaction can also be obtained with pluroglucin-Hcl. Evidently the bacteria secrete an enzyme which dissolves the soluble pectins of the middle lamella and after these are made available to

the bacteria a residue is left. This residue is the yellow to brown discoloration which is such a characteristic symptom of diseased roots. It differs from the gum-like material found in the invaded vessels, as it is water-soluble and can apparently spread into the root tissues.

LeClerg and Durrell (9) have also noted that plugging of the vessels is accompanied by a change in the composition of the walls of groups of cells surrounding them, as indicated by a brilliant reaction to pluroglucin-Hcl, although they offer no explanation of its underlying cause. Jones (5) also describes in detail the gum in the vessels and the root discoloration, due to the yellow soluble material which, he says, diffuses to a considerable distance beyond the bacterial invasion. Both LeClerg and Durrell (9) and Jones (5) state that the gum-like material formed in invaded vessels is a product of the alfalfa plant and not of the bacteria. The writers are inclined to believe that both the insoluble gum in the vessels and the soluble material produced in the parenchymatous tissues are residues of bacterial action on the soluble pectins present in these tissues.

In examining sections of a number of plants inoculated in May and fixed in October and again in April, it was noted that the vessels formed in the late fall and the vessels formed first in the spring did not take the safranin stain but remained colorless. When sections of this material were tested with pluroglucin-Hcl a negative reaction of the vessel walls was obtained while with ruthenium red a positive reaction occurred. These results were checked repeatedly with similar sections from a number of different alfalfas. This gives us a clue as to how the bacteria migrate from the last-formed vessels of the fall wood where presumably they remain latent during the winter, to the first-developed vessels of the spring wood. Jones (5) states that "bacteria develop more abundantly both in the parenchymatous tissue and in the vessels of wood produced in the autumn." He interprets this "as indicating a high degree of susceptibility of autumn wood to bacterial invasion in comparison with summer wood." He seems to believe that "it is associated with the comparative absence of fibers and that it may be due to physical or chemical characteristics of the cell walls." He concludes that "Whatever its nature, it is obvious that if the alfalfa plant did not produce so much autumn wood in the root and crown it might be highly resistant to the disease."

It is true that Turkestan plants go into dormancy much earlier in the fall than many susceptible alfalfas. Grimm, however, has a tendency toward early dormancy, yet it is extremely susceptible to wilt, so that we must look for other

causes. Fall wood is much more susceptible to wilt than summer wood, but it is not more susceptible than spring wood. In the spring conditions are usually ideal for rapid growth. During the summer in Nebraska high temperatures, hot winds, and lack of moisture curtail the growth of the plants. As a rule the yields of the second and third cuttings are smaller than those of the first. Therefore it is not surprising that the summer wood is generally more resistant to invasion, primarily because of the slower growth of the plants under these unfavorable conditions.

In the fall with the gradual shortening of the days and progressively lower mean temperatures, the plants slowly go into dormancy. This takes place over a rather long period and varies with the kind of alfalfa (14). While the vessels being laid down are of smaller diameter, their period of maturation is longer, so that the bacteria are able to develop in the fall wood. The young vessels formed just before complete dormancy occurs do not mature and if infected with bacteria they remain latent in those vessels during the winter. In the spring when new growth is initiated they are able to make an exit at the pits through the still soluble middle lamella and invade the newly-formed vessels of the spring wood. If the bacteria do not succeed in making an exit, because of the lignification of the vessel pits or the infiltration of the middle lamella with calcium to form an insoluble calcium pectase, they are trapped and the newly developed wood remains uninfected. No proof has yet been found to show that the bacteria in the intercellular spaces of parenchymatous tissue remain latent during the winter and are the source of infection in the spring wood. As a rule plants in which bacteria are present in the parenchymatous tissues in the fall do not survive the winter or spring because of the small amount of root reserves found in plants in this stage of disease development. Unless the bacteria succeed in invading the newly-formed vessels of the spring wood, they remain segregated in the previous year's wood and the plant may recover.

LITERATURE CITED

1. Dixon, H. H., and N. G. Ball
1923. TRANSPORT OF ORGANIC SUBSTANCES IN PLANTS. Notes Bot. School Trinity College, Dublin, 3:207-215.
2. Graber, L. F., N. T. Nelson, W. A. Leukel, and W. B. Albert
1927. ORGANIC FOOD RESERVES IN RELATION TO THE GROWTH OF ALFALFA AND OTHER PERENNIAL HERBACEOUS PLANTS. Wisc. Agr. Expt. Sta. Res. Bul. 80, 128 pp., illus.
3. Jones, F. R.
1925. A NEW BACTERIAL DISEASE OF ALFALFA. Phytopathology, 15:243-244.

4. ————————
1928. WINTER INJURY OF ALFALFA. Jour. Agr. Research, 37:189-211, illus.

5. ————————
1928. DEVELOPMENT OF THE BACTERIA CAUSING WILT IN THE ALFALFA PLANT AS INFLUENCED BY GROWTH AND WINTER INJURY. Jour. Agr. Research, 37:545-569, illus.

6. ————————
1930. BACTERIAL WILT OF ALFALFA. Jour. Amer. Soc. Agron., 22:568-572.

7. ———————— and Lucia McCulloch
1926. A BACTERIAL WILT AND ROOT ROT OF ALFALFA CAUSED BY APLANOBACTER INSIDIOSUM *L. McC.* Jour. Agr. Research, 33:493-521, illus.

8. ———————— and J. L. Weimer
1928. BACTERIAL WILT AND WINTER INJURY OF ALFALFA. U. S. Dept. Agr. Circ. 39, 8 pp., illus.

9. LeClerg, E. L., and L. W. Durrell
1928. VASCULAR STRUCTURE AND PLUGGING OF ALFALFA ROOTS. Colo. Agr. Expt. Sta. Bul. 339, 19 pp., illus.

10. McCulloch, Lucia
1925. APLANOBACTER INSIDIOSUM N. SP. THE CAUSE OF AN ALFALFA DISEASE. Phytopathology, 15:496-497.

11. Peltier, G. L.
1920. INFLUENCE OF TEMPERATURE AND HUMIDITY ON THE GROWTH OF PSEUDOMONAS CITRI AND ITS HOST PLANTS AND ON INFECTION AND DEVELOPMENT OF THE DISEASE. Jour. Agr. Research, 20:447-506.

12. ———————— and J. H. Jensen
1930. ALFALFA WILT IN NEBRASKA. Nebr. Agr. Expt. Sta. Bul. 240, 30 pp., illus.

13. ———————— and H. M. Tysdal
1930. THE RELATIVE SUSCEPTIBILITY OF ALFALFAS TO WILT AND COLD. Neb. Agr. Expt. Sta. Res. Bul. 52, 15 pp.

14. ————————
1931. HARDINESS STUDIES WITH 2-YEAR-OLD ALFALFA PLANTS. Jour. Agr. Research, 43:931-955, illus.

15. Salmon, S. C.
1930. THE REACTION OF ALFALFA VARIETIES TO BACTERIAL WILT. Jour. Amer. Soc. Agron., 22:802-810.

16. Shantz, H. L., and L. M. Piemeisel
1927. THE WATER REQUIREMENTS OF PLANTS AT AKRON, COLORADO. Jour. Agr. Research, 34:1093-1190, illus.

17. Society of American Bacteriologists, Committee on Bacteriological Technic
1930. MANUAL OF METHODS FOR PURE CULTURE STUDY OF BACTERIA. 50 pp., Geneva, N. Y.

18. Wolf, F. A.
1923. STUDIES ON THE PHYSIOLOGY OF SOME PATHOGENIC BACTERIA VII—PECTIC FERMENTATION IN CULTURE MEDIA CONTAINING PECTIN. Phytopathology, 13:381-384.

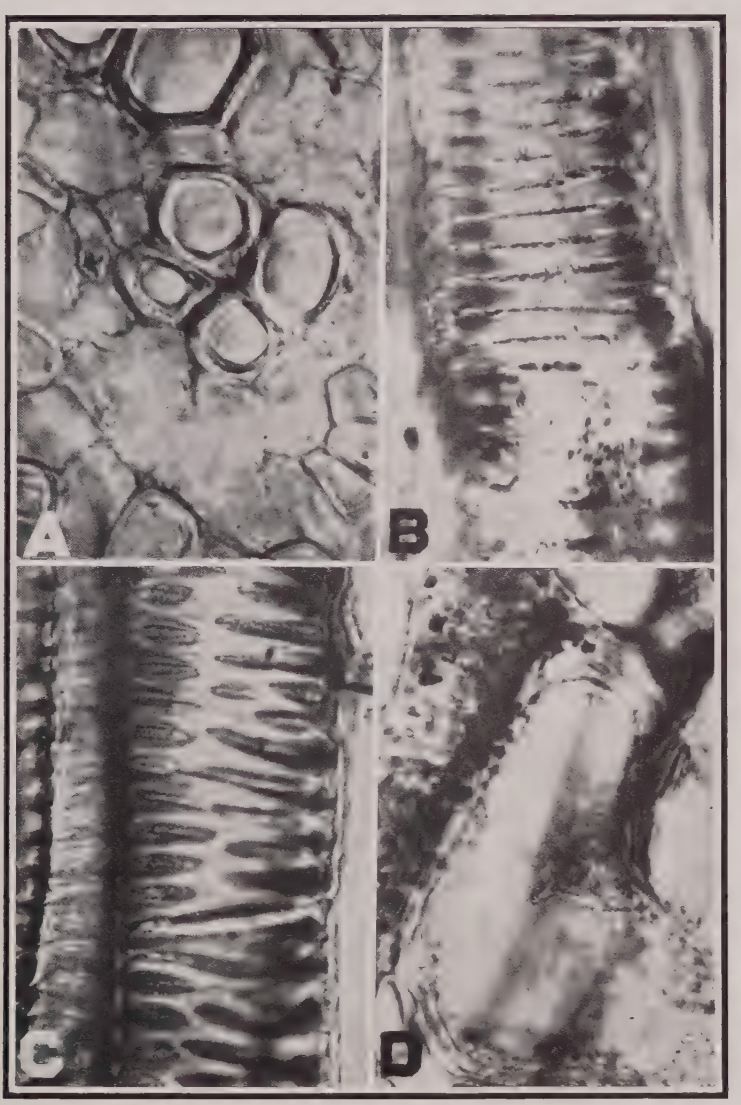

PLATE 1.—A.—A stained root cross section of a Grimm (F. C. I. No. 15936) plant, inoculated at the age of three weeks and fixed four weeks later. Bacteria are present in the middle lamella between vessels. This shows an early stage in the entrance of the bacteria into the vessels. X 1070.

B.—A stained root longitudinal section of an Arizona Common (F. C. I. No. 15837) plant inoculated at the age of ten weeks and fixed three months later. Bacteria are present in the lumen of the lower vessel segment. In the upper vessel segment bacteria are fewer and are almost entirely confined to the pits. The membrane of the pits in which no bacteria are present do not appear to be homogeneous but more like minutely reticulated membranes. X 1070.

C.—Section of a Turkestan (F. C. I. 15754) plant, similar to B. The reticulated nature of the pit membranes is clearly evident. X 1070.

D.—A stained longitudinal root section of a Turkestan (F. C. I. No. 15754) plant inoculated at the age of six weeks and fixed four months later. Bacteria have broken out of the vessels through a pit and are present in the intercellular spaces of parenchyma cells adjacent to the vessels. X 640.

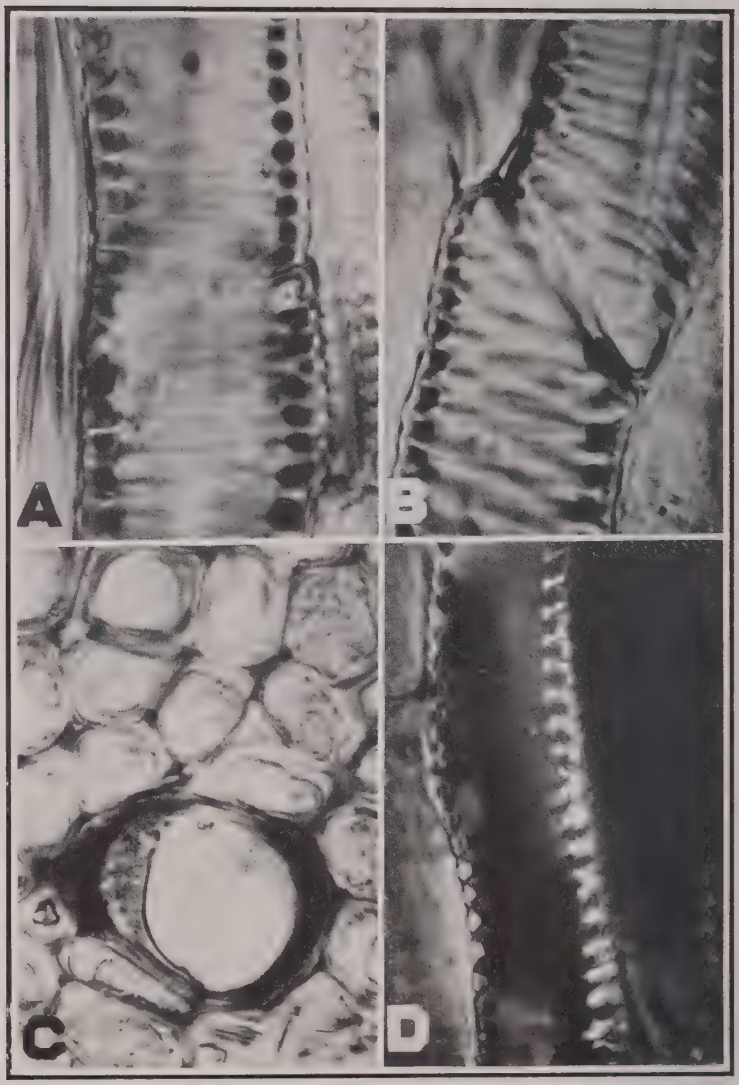

PLATE 2.—A.—A stained longitudinal root section of a Grimm (F. C. I. No. 15936) plant, fixed when the plant was about six months old. The end walls between the vessel segments are very inconspicuous, allowing for the unhampered passage of the bacteria in the vessels. X 1070.

B.—A stained longitudinal root section of a Turkestan (F. C. I. No. 15754) plant fixed when the plant was about six months old. Secondary and tertiary thickenings of the incomplete end wall segments form partial barriers or dams behind which the bacteria drift and accumulate before entering the next vessel segment. X 1070.

C.—A cross section of the same plant as in B, showing an end wall of the vessel segments which clearly shows the shelf-like structure or dam projecting out into the lumen of the vessel. X 1070.

D.—A longitudinal section of the two vessels as in B. Note the exact opposition of the pits of the two contiguous vessels. X 1070

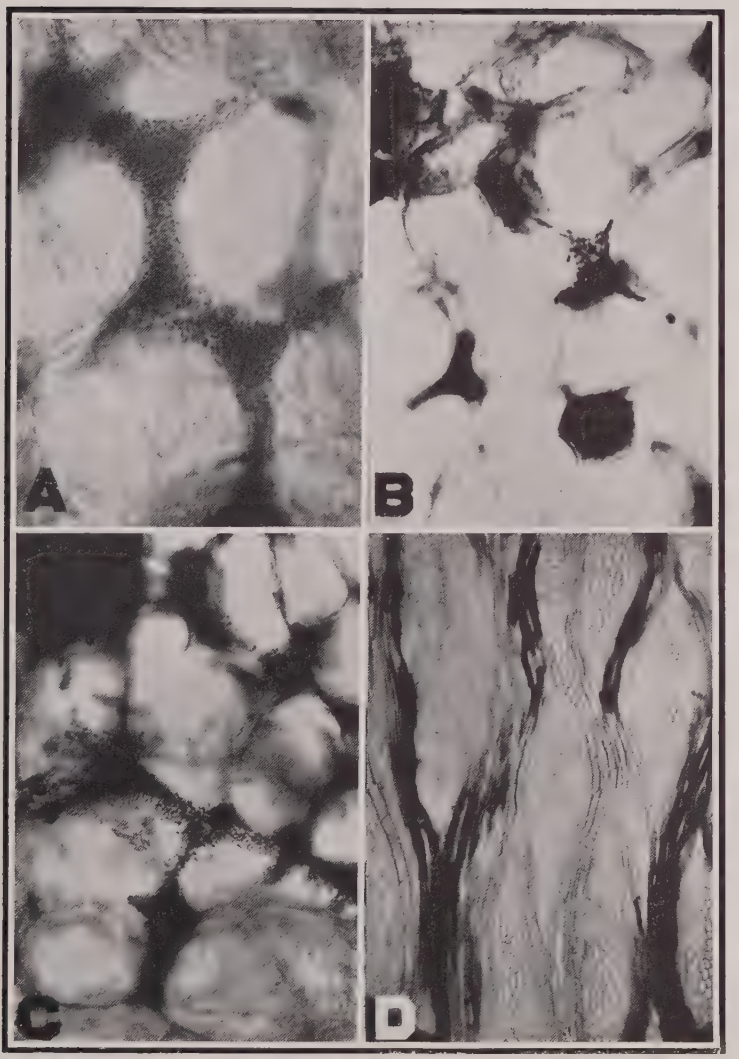

PLATE 3.—Portions of stained root cross sections of Grimm, Arizona Common, and Turkestan plants inoculated at the age of three weeks and fixed two months later. The bacteria are present in the intercellular spaces of the parenchyma cells of the medullary rays and extend along the middle lamella. Some of the intercellular spaces are filled with bacteria and soluble gum which readily take up safranin stain.

A.—Grimm, X 1070.

B.—Arizona, X 850.

C.—Turkestan, X 640.

D.—A stained tangential root section of an Arizona Common (F. C. I. No. 15837) plant inoculated at the age of ten weeks, grown under optimum soil moisture until fixed three months later. The dark masses in the vessels are bacteria and gum, which is formed as the result of bacterial invasion. The anastomosing vessels make it possible for the bacteria to be transported from one vessel to another of the same age around the circumference of the root, after the bacteria pass through the contiguous pits of the vessels, in exact opposition. X 85.

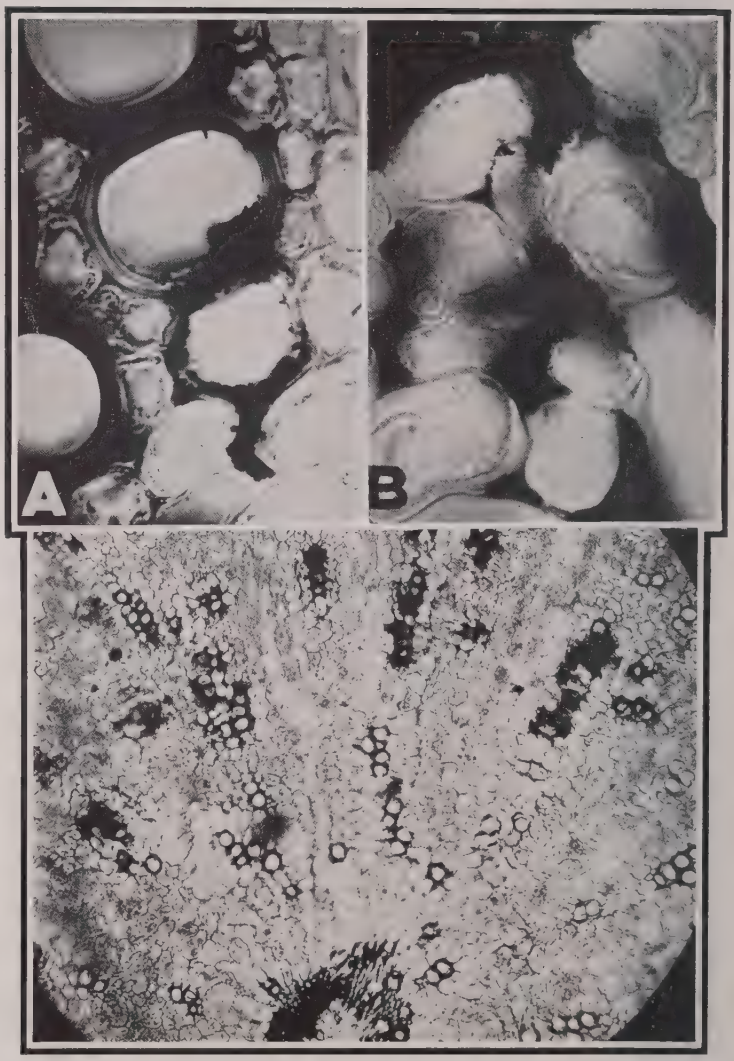

PLATE 4.—A.—Portion of a cross section of a stem from a severely wilted plant of Grimm. The bacteria can be seen migrating between the intercellular spaces of the parenchyma cells to vessels. Entrance through the pits is just being effected. X 1070.

B.—Another portion of the section showing the migration of the bacteria through the intercellular spaces and middle lamellae of the parenchymatous tissue. Large masses of bacteria are present in some of the intercellular spaces. X 1070.

C.—(Lower cut.) A stained root section of a Turkestan (F. C. I. No. 15754) plant, inoculated at the age of four weeks and fixed three months later. Bacterial invasion of some vessels occurred at the time of inoculation at the center of the section, but no further progress was made into other developing vessels. A discoloration of the peripheral vessels, however, was apparent in an unstained glycerin mount. On staining the cells of the discolored areas had a tendency to retain the stains more readily than did other cells. This staining reaction of the discolored cells can be considered as due to the chemical nature of the cell walls. The yellow soluble discolorations usually found on peeling the bark away from the woody cylinder of diseased plants seems to diffuse out considerably in advance of the bacteria. X 160.

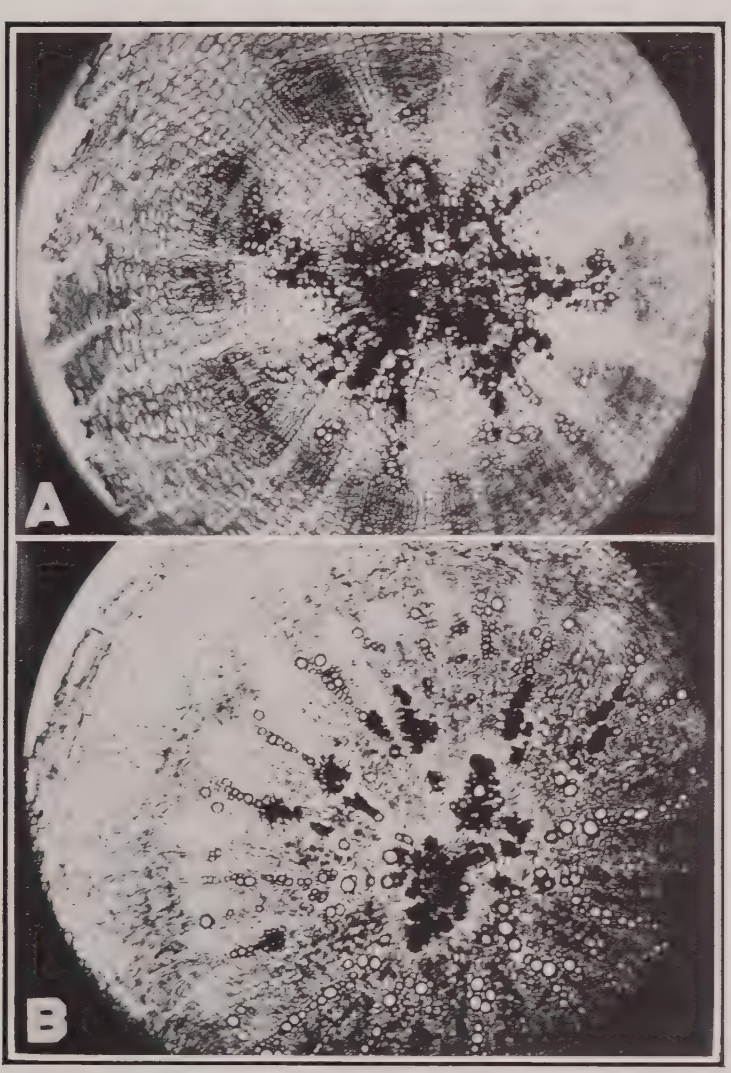

PLATE 5.—Stained root cross sections of two alfalfas inoculated by the root-severance method under a bacterial suspension when three weeks old and grown under identical conditions until fixed two months later. The difference in the extent of invasion between a susceptible and a resistant sort is apparent.

A.—Grimm (F. C. I. No. 15936). Entry occurred in the first-formed vessels and continued in the outer vessels as they developed. The plant was almost dead at the time of fixation. Note bacteria filling some of the intercellular spaces in the medullary rays and the complete absence of starch in these cells and the decided dwarfing of the root. X 80.

B.—Turkestan (F. C. I. No. 15754). Entry occurred in the first-formed vessels. Invasion of the other vessels as they were developing was not continuous. Note that the vessels nearest the cambium do not contain bacteria. The plant exhibited only slight top symptoms at the time of fixation. There are relatively few bacteria in the intercellular spaces of the medullary rays. X 80.

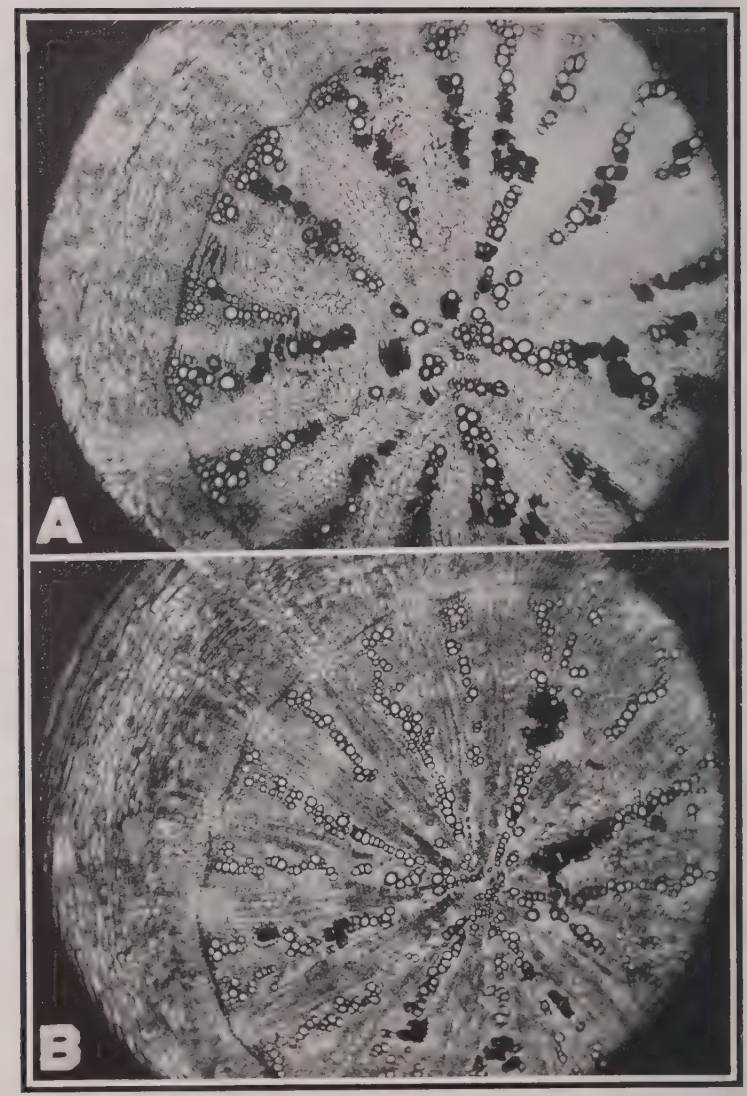

PLATE 6.—Stained root cross sections of two alfalfas inoculated by the root-severance method under a bacterial suspension, when 12 weeks old and grown under identical conditions until fixed two months later. The difference in extent of invasion of the vessels laid down subsequent to inoculation is apparent.

A.—Arizona Common (F. C. I. No. 15837). At time of inoculation the bacteria entered and developed in the vessels a considerable distance from the center. Many vessels around the same circumference were invaded, and the invasion of new vessels was more or less continuous. Note the bacteria in the intercellular spaces of the medullary ray cells and the complete absence of starch in all these cells. X 60.

B.—Turkestan (F. C. I. No. 15754). Several vessels in groups in some of the bundles show the characteristic plugging by the bacteria and gum. Starch is present in the medullary rays adjacent to healthy bundles. Few bacteria are to be seen in the intercellular spaces of the medullary ray cells. X 60.

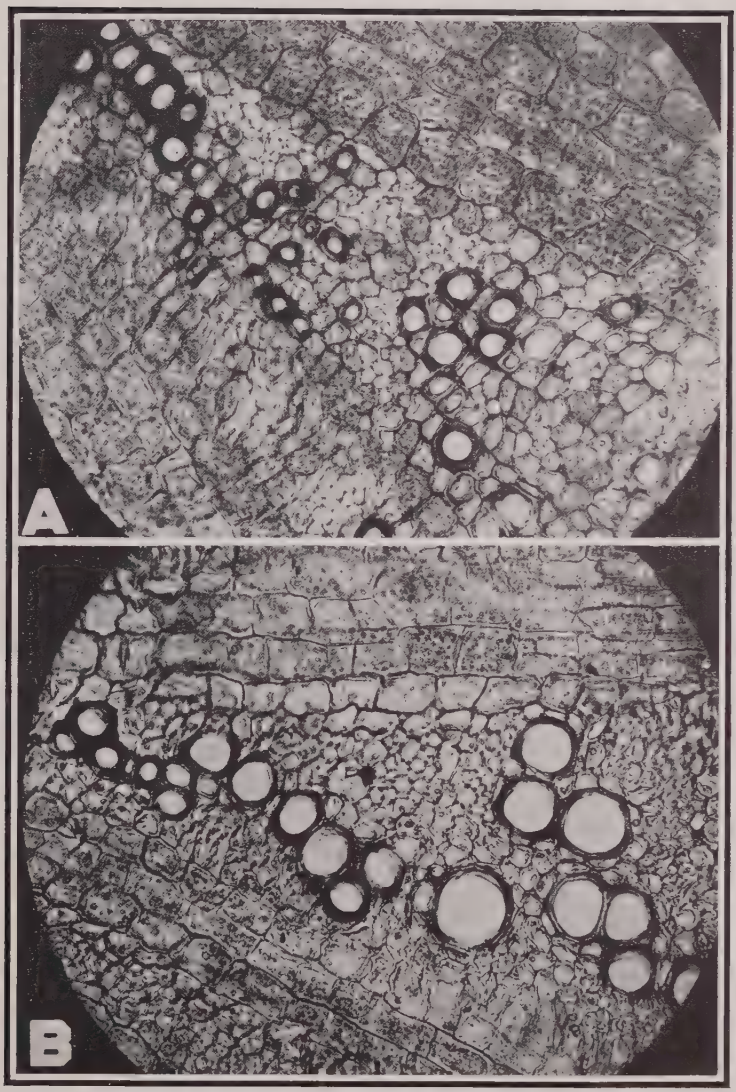

PLATE 7.—Stained cross sections of comparable portions of radial bundles of roots of healthy plants grown under identical conditions for five months before being fixed.

A.—Turkestan (F. C. I. No. 15754). The vessels are small in diameter, angular, and with secondary and tertiary thickenings. Note the disconnected arrangement of the vessel groups in the bundle, together with the number and location of the wood fibers with respect to the vessels and medullary ray cells. An occasional wood parenchyma cell can be found interspersed among the wood fibers. The parenchyma cells of the medullary rays are compactly arranged and rather thick walled. X 210.

B.—Arizona Common (F. C. I. No. 15837). The vessels are large in diameter, rounded, with comparatively thin secondary and partial tertiary thickenings. Note the more or less contiguous arrangement of the vessels in the bundle, together with the small number and location of the wood fibers with respect to the vessels and medullary ray cells. Many wood parenchyma cells can be found interspersed among the wood fibers and immediately around the vessels. The parenchyma cells of the medullary rays are rather loosely arranged and rather thin walled. X 210.

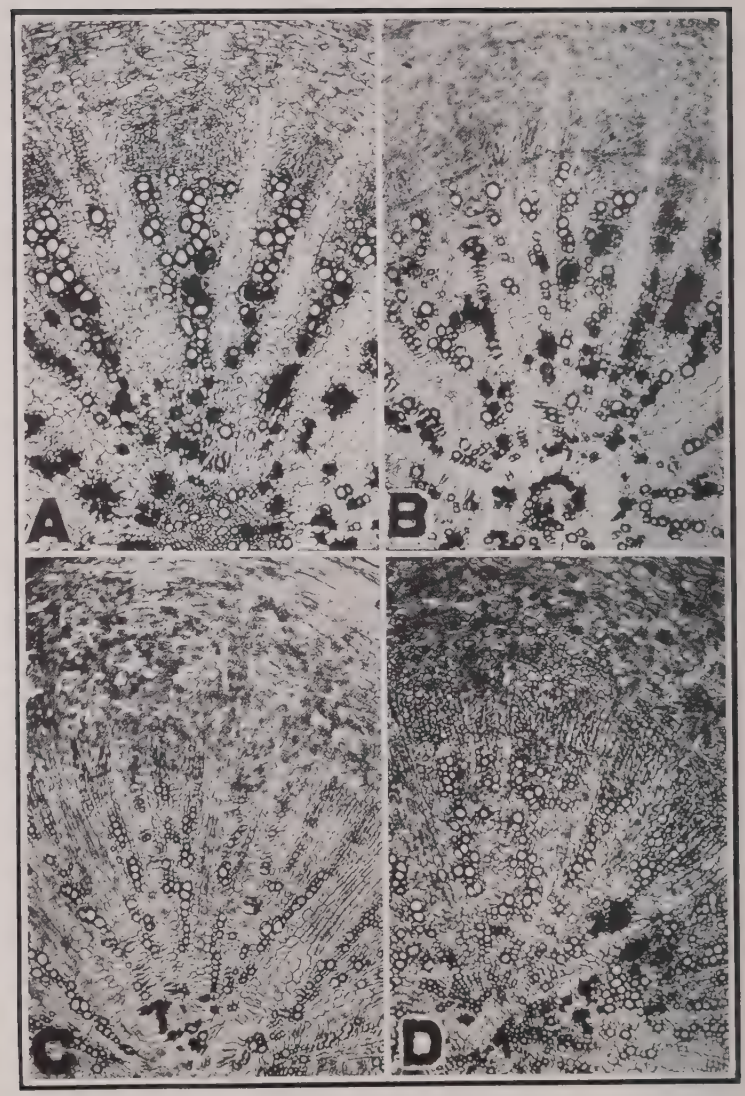

PLATE 8.—Stained root cross sections inoculated at the age of three weeks and grown under controlled conditions for two and one-half months in the greenhouse before fixation. Note the structure of the susceptible and resistant sorts.

A.—A susceptible alfalfa, from Zaragosa, Spain (F. P. I. No. 89846). This sort showed 100 per cent susceptibility in greenhouse tests. X 80.

B.—A susceptible alfalfa from New Mexico. Seed obtained from an old field supposedly resistant to wilt. This alfalfa showed 98 per cent susceptibility in greenhouse tests. X 80.

C.—An alfalfa from Ashkhabad, South Turkestan (F. C. I. No. 19304). This alfalfa showed 30 per cent susceptibility in greenhouse tests. X 50.

D.—From an old field in South Dakota (F. C. I. No. 19299). Supposedly Turkestan. This sort showed 47 per cent susceptibility in greenhouse tests. X 80.

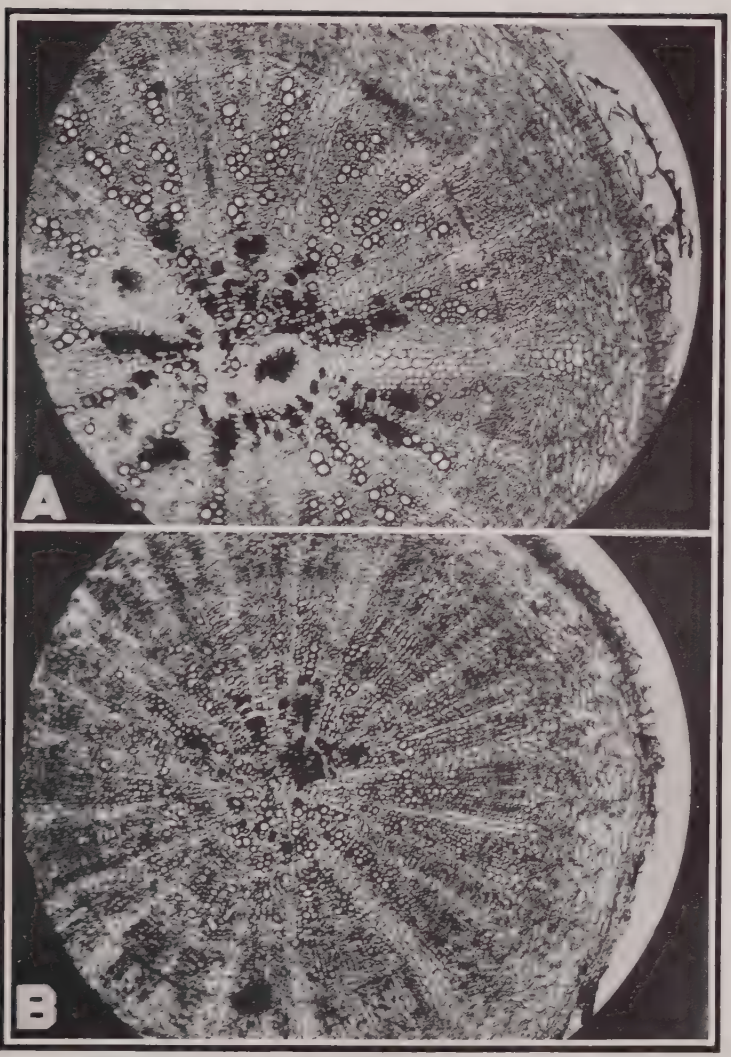

PLATE 9.—Stained root cross sections of alfalfa (Assadabad, Persia, F. C. I No. 26362) plants, inoculated at the age of three weeks and grown for two and one-half months prior to fixation. Note the structure of the susceptible and resistant types within the same sort of alfalfa. X 30.

A.—Susceptible type.

B.—Resistant type.

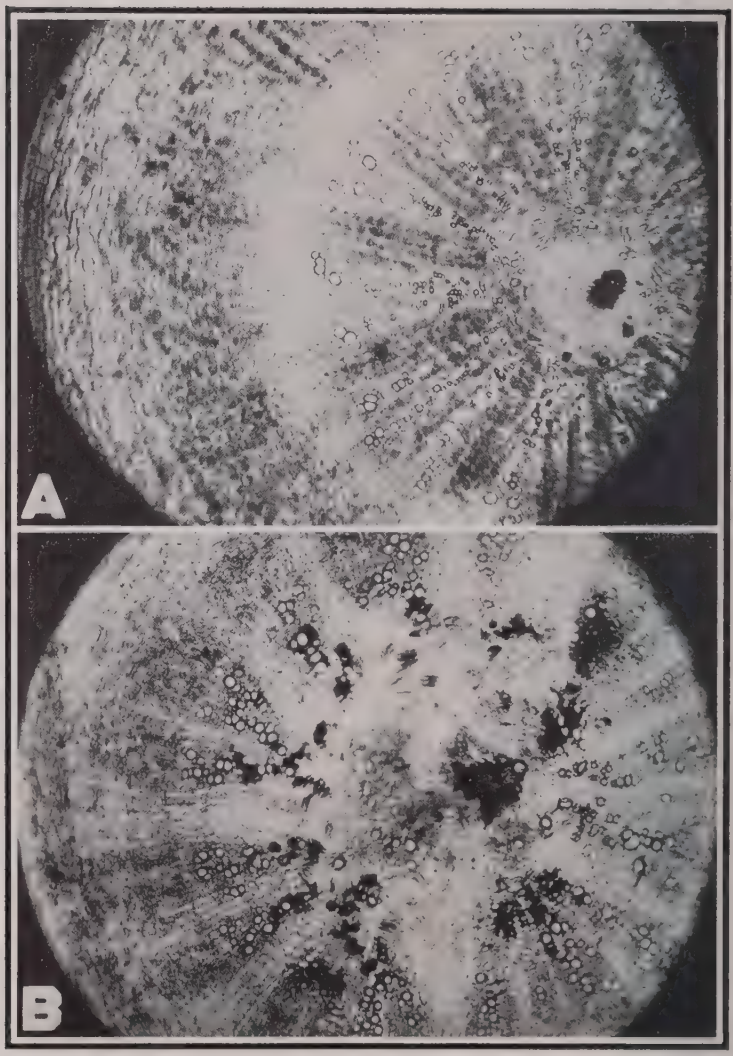

PLATE 10.—Stained root cross sections of Turkestan (F. C. I. 15754) plants, inoculated at the age of one month and grown under different soil moisture conditions for four months before being fixed.

A.—Grown under low soil-moisture conditions. A few of the first-formed vessels are filled with bacteria and gum. Starch is absent in a definite area of the parenchymatous tissue surrounding the infected vessels. The vessels are small in diameter and surrounded by wood fibers. The plant after initial infection remained healthy as denoted by the large amount of starch in the parenchymatous tissue in the uninvaded areas. X 75.

B.—Grown under high soil-moisture conditions. Bacteria and gum are present in a number of vessels of the radial bundles. The vessels are rather large in diameter and wood fibers are not so conspicuous. Starch is absent in most of the medullary ray cells adjacent to infected bundles. Dwarfing is evident. X 75.

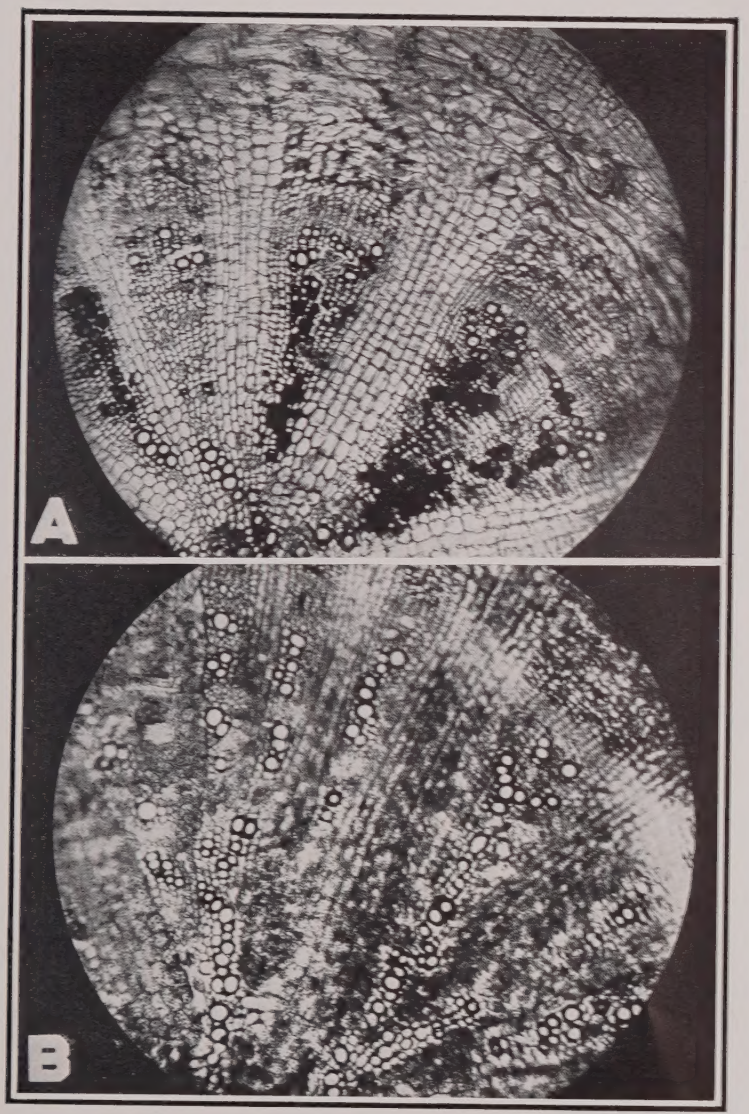

PLATE 11.—Stained root cross sections of Grimm (F. C. I. No. 15936) plants. One was inoculated at the age of ten weeks, grown under optimum soil-moisture conditions and fixed at the end of five and one-half months. The second was a control plant grown under identical controlled conditions.

A.—Bacteria and gum fill most of the vessels in the radial bundles. Bacteria can be seen in the intercellular spaces of the parenchyma cells. Note the complete absence of starch in all the parenchyma cells and the decided dwarfing of the root. X 75.

B.—The parenchyma cells of the wood and cortex are gorged with starch. X 75.

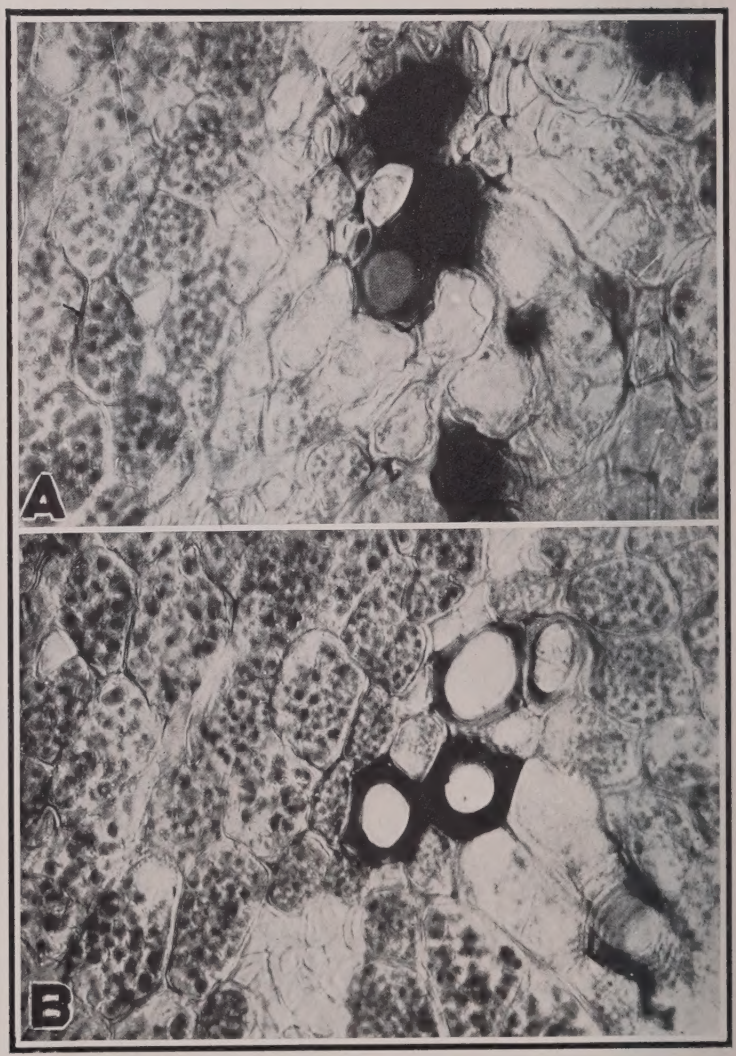

PLATE 12.—Portions of a stained root cross section of a Grimm (F. C. I. No. 15936) plant, inoculated at the age of ten weeks and fixed three and one-half months later.

A.—Two vessels completely filled with bacteria and gum which stains an intense red with safranin. Note that starch is absent from the parenchyma cells immediately surrounding the invaded vessels. Below to one side in the intercellular spaces is a yellow discoloration which also takes a stain with safranin. X 400.

B.—Several healthy vessels approximately an equal distance inward from the cambium in an adjacent bundle. The parenchyma cells surrounding these vessels are completely gorged with starch. X 400.